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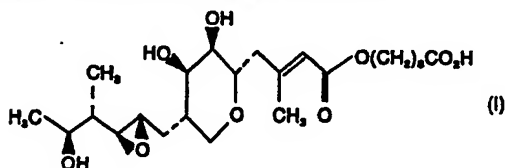
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36 Silver pseudomonic acid, compositions containing it and its use in treating pseudomonal infections.

37 Pseudomonic acid (I) is an antibiotic produced by aerobically culturing *Pseudomonas fluorescens*.



A process is provided for producing silver pseudomonic acid which process comprises reacting silver ions and pseudomonic acid or pseudomonic ions in aqueous solution and thereafter recovering the silver pseudomonic acid so formed.

Also provided is a method for treating wounds or burns infected with *Pseudomonas* organisms comprising administering a non-toxic anti-pseudomonally effective amount of silver pseudomonic acid to the wound or burn.

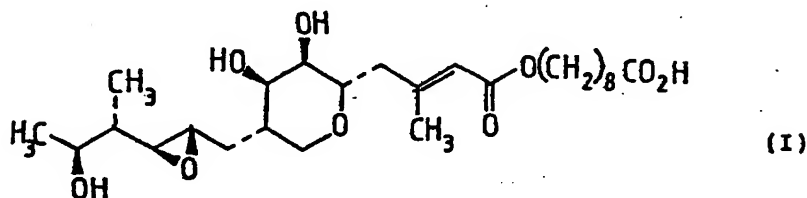
EP 0 128 338 A1

TITLE MODIFIED
see front page

COMPOUND AND USE

The present invention relates to silver pseudomonate, compositions containing it and its use in treating pseudomonal infections.

Pseudomonic acid is an antibiotic produced by aerobically culturing Pseudomonas fluorescens. The compound, of formula (I) below, and its salts and esters are disclosed and claimed in UK Patent No. 1 395 907.



Whilst pseudomonic acid and its salts and esters are active against a variety of human and animal pathogens (see for instance UK Patent Nos. 1 577 730 and 1 577 545), they are not active at useful levels against Pseudomonas species.

Pseudomonas organisms tend to infect burns and wounds. Such infections are often difficult to treat as the organisms are not particularly susceptible to antibiotics.

01
02 It has now surprisingly been found that silver
03 pseudomonate is active against Pseudomonas organisms,
04 especially Pseudomonas aeruginosa, the causative agent
05 of 'blue pus' infections.
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07 The silver salt of pseudomonic acid has not been
08 specifically disclosed in the above patents or any
09 other publications and is, therefore, novel.
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11 Accordingly the present invention provides, in one
12 aspect, silver pseudomonate.
13

14 The invention also provides silver pseudomonate
15 for use in the treatment of the human or animal body.
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17 Apart from its surprising activity against
18 Pseudomonas, silver pseudomonate has a similar spectrum
19 of activity against pathogens to those of pseudomonic
20 acid and sodium pseudomonate.
21

22 Accordingly the present invention also provides
23 silver pseudomonate for use in treating the human or
24 animal body, especially for treating infected wounds
25 and burns.
26

27 The invention also provides a process for
28 producing silver pseudomonate which process comprises
29 reacting silver ions and pseudomonic acid or
30 pseudomonate ions in aqueous solution and thereafter
31 recovering the silver pseudomonate so formed.
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33 Suitably the process is effected by adding a
34 source of silver ions to an aqueous solution of
35 pseudomonic acid or a pseudomonate salt, especially
36 sodium pseudomonate.
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02 Suitably the solution of pseudomonic acid or
03 pseudomonte ions is the product of aerobically
04 culturing Pseudomonas fluorescens (NCIB 10586). Such a
05 solution may be the culture medium in which the
06 organisms have been grown or it may have been produced
07 by purifying such a medium for instance by extracting
08 pseudomonic acid from such a culture medium using a
09 polar, organic, water-immiscible solvent as described
10 in EP 0 005 614. Alternatively the solution of
11 pseudomonic acid or pseudomonte ions may be produced
12 by dissolving pseudomonic acid or preferably a salt
13 thereof, in an aqueous solvent. Preferably the
14 solution is produced by dissolving pure sodium
15 pseudomonte in water.

16
17 The source of silver ions is preferably a soluble
18 silver salt such as silver nitrate or silver carbonate.

19
20 The invention further provides silver pseudomonte
21 in substantially pure form, preferably at least 75%
22 pure, more preferably at least 90% pure, most
23 preferably at least 95% pure.

24
25 If precipitated from solution containing solvents
26 other than water, the silver pseudomonte may be
27 produced in a solvated form including a hydrated form.
28 If precipitated from aqueous solution the silver
29 pseudomonte may be in a hydrated form.

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31 Accordingly the invention further provides
32 solvated, including hydrated, silver pseudomonte.

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34 Silver pseudomonte may be administered as the
35 pure compound (hereinafter referred to as the 'drug')
36 or it may be administered as a pharmaceutical
37 composition in association with a suitable carrier.

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Accordingly the invention also provides a pharmaceutical formulation comprising silver pseudomonate and a pharmaceutically acceptable carrier therefor.

As used herein the term 'pharmaceutically acceptable' includes 'veterinarily acceptable'.

The formulations may be adapted for administration by any route, and would depend on the disease being treated. Normally, the formulations will be presented as topical solutions or suspensions for application to the skin, ears or eyes. Alternatively the formulations may be dry powders for application as an aerosol, or they may be presented as impregnated dressings for wounds and burns.

For topical application to the skin the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations that may be used for the drug are conventional formulations well known in the art, for example, as described in standard text books of pharmaceutics and cosmetics, such as Harry's Cosmeticology published by Leonard Hill Books, and the British Pharmacopoeia. Alternatively the drug may be applied as a dry powder from an aerosol using conventional diluents and propellants.

For topical application to the ear, the drug may be made up into a solution or suspension in a suitable liquid carrier, such as water, glycerol, diluted ethanol, propylene glycol, polyethylene glycol or fixed oils.

For topical application to the eye, the drug is formulated as a solution or suspension in a suitable, sterile aqueous or non-aqueous vehicle. Additives, for

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02 instance buffers such as sodium metabisulphite or
03 disodium edetate; preservatives including bactericidal
04 and fungicidal agents, such as phenylmercuric acetate
05 or nitrate or chlorhexidine, and thickening agents such
06 as hypromellose may also be included.

07

08 Particularly suitable topical formulations
09 comprise silver pseudominate and at least 1% by weight
10 of a poly (substituted or unsubstituted alkylene)
11 glycol or a derivative thereof.

12

13 As used herein the term 'poly (substituted or
14 unsubstituted alkylene) glycol' refers to polymers
15 having the following repeating unit

16



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19 wherein n is an integer, preferably 2 or 3 and to such
20 polymers wherein one or more methylene groups of each
21 repeating unit is substituted. Suitable substituents
22 include alkoxy groups such as methoxy as in
23 polymethoxypropylene glycol. Such polymers are known
24 by a variety of names, for instance when n = 2, as
25 polyethylene glycol, polyoxyethylene, polyoxyethylene
26 glycol and macrogol and, when n = 3, as polypropylene
27 glycol, polyoxypropylene and polyoxypropylene glycol.
28 All these are useful in the invention as are
29 derivatives of these polymers.

30

31 Suitable derivatives include ethers and esters of
32 the poly (substituted or unsubstituted alkylene)
33 glycols, such as the macrogol ethers and esters, for

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02 instance cetomacrogol, glycofurol, the 'Tweens'* and
03 block copolymers including poly (substituted or
04 unsubstituted alkylene) glycols such as Poloxamers
05 which are block copolymers of polyethylene glycol and
06 polypropylene glycol for instance the 'Pluronics'*, and
07 cross-linked polyethylene glycol.

08

09 The poly (substituted or unsubstituted alkylene)
10 glycols and derivatives thereof may be used singly or
11 various grades and types may be used in combination to
12 achieve the desired physical properties of the
13 formulation.

14

15 Preferably the formulation comprises polyethylene
16 glycol or a derivative thereof.

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18 Suitably the formulation comprises from 0.01 to
19 50% by weight of silver pseudomonte, preferably 0.1 to
20 25%, more preferably 0.5 to 10% and most preferably
21 about 2% by weight of silver pseudomonte calculated as
22 the free acid. Such formulations comprising only
23 silver pseudomonte and a poly (substituted or
24 unsubstituted alkylene) glycol or derivative thereof
25 will, of course, contain up to 99.99% of the poly
26 (substituted or unsubstituted alkylene) glycol or
27 derivative thereof.

28

29 The formulation may comprise additional
30 therapeutic agents such as antibacterial, antifungal,
31 antiviral and antiinflammatory agents, for instance
32 chlortetracycline, miconazole, idoxuridine and
33 phenazone, provided that these are compatible with the

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35 * 'Tween' and 'Pluronic' are trade names for the above
36 types of polymer.

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silver pseudomonte. Silver Pseudomonte tends to undergo a rearrangement reaction in the presence of acid and accordingly acidic agents are unlikely to be compatible with silver pseudomonte.

In a particular aspect the invention provides a topical formulation as described above wherein silver pseudomonte is the sole therapeutic agent.

In another aspect the invention provides a topical formulation comprising silver pseudomonte and at least 1% by weight of polyethylene glycol or a derivative thereof.

Polyethylene glycols (PEG's) and derivatives thereof are commercially available in a variety of chain lengths and with a variety of consistencies, for instance:-

Polyethylene Glycols:-

Liquids	Semisolids	Hard Solids
PEG 200 PEG 300 PEG 400	PEG 1000 PEG 1540	PEG 4000* PEG 6000

Polyethylene Glycol derivatives:-

Derivative	Chemical Composition	Consistency
Glycofurol	Tetrahydrofurfuryl alcohol polyethylene glycol ether	Liquid
Tween 60	Polyoxyethylene Sorbitan monostearate	Semi-solid
Tween 80	Polyoxyethylene Sorbitan. monooleate	Liquid

* PEG 4000 is the B.P. nomenclature for PEG with mean molecular weight of 3350. This material is also known as PEG 3350 in U.S.P. nomenclature.

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These may be used singly or admixed in suitable proportions to achieve the desired consistency of formulation.

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The formulations of the present invention may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams. The formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

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Particularly suitable formulations according to the present invention comprise at least 1% by weight of PEG or a mixture of PEG's, from 0 to 25% by weight of a PEG derivative or mixture of PEG derivatives and from 0.5 to 10% by weight of silver pseudomonate calculated as the free acid.

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Preferably the silver pseudomonate represents 1 to 5% of the formulation, most preferably about 2% of the formulation calculated as the free acid.

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Formulations of the invention may be produced by conventional pharmaceutical techniques. Thus ointments and creams are conveniently prepared by melting and mixing together the solid or semi-solid PEG's or PEG analogues or derivatives, and stirring in the therapeutic agent and any other ingredients. The product is then slowly cooled and filled into containers such as collapsible metal or plastic tubes.

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02 Liquid preparations, such as ear and eye drops,
03 are produced by dissolving the therapeutic agent in the
04 liquid PEG's or PEG analogues or derivatives and the
05 other ingredients are then added. The resulting
06 solution or suspension is distributed into glass or
07 plastic bottles or in single dose packs such as soft
08 gelatin capsules which are then heat sealed.

09
10 If necessary the formulation may be milled at any
11 suitable stage of the process.

12
13 A suitable sterilisation procedure may be included
14 in the above processes if necessary. Alternatively raw
15 materials are obtained in sterile conditions and the
16 formulations are produced aseptically.

17
18 The dosage employed for formulations administered
19 topically will, of course, depend on the size of the
20 area being treated. For the ears and eyes each dose
21 will typically be in the range from 10 to 100 mg of the
22 drug.

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24 The present invention further provides a process
25 for producing a pharmaceutical formulation which
26 process comprises bringing into association silver
27 pseudomonate and a pharmaceutically acceptable carrier
28 therefor.

29
30 The present invention also provides a method for
31 treating pseudomonal infections of human or non-human
32 animals comprising administering a non-toxic
33 anti-pseudomonally effective amount of silver
34 pseudomonate to an infected human or non-human animal.
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In a particular aspect the invention provides a method for treating wounds or burns infected with Pseudomonas organisms comprising administering a non-toxic anti-pseudomonally effective amount of silver pseudomonate to the wound or burn.

Preferably the above methods are effected by applying a topical formulation to the infected area.

The invention will now be illustrated with reference to the following Examples and Biological data.

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Example 1

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Silver Pseudomonate A

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Sodium pseudomonate A (1.82g, 4 mmol) and silver nitrate (0.68g, 4 mmol) were stirred in distilled water for 30 min resulting in the formulation of a white gelatinous precipitate. The mixture was centrifuged, the aqueous layer removed and the residue washed with distilled water. The suspension was centrifuged and the residual solid was dried over phosphorus pentoxide under high vacuum for 2 days to yield silver pseudomonate A, m.p. 164-166°C, (855 mg, 35%); $\nu_{\max}(\text{KBr})$ 3400, 1710, 1645, 1515 cm^{-1} ; $\delta_{\text{H}}(\text{CD}_3)_2\text{SO}$ 5.68 (1H, s, H2), 2.12 (3H, s, CH₃-15), 1.1 (3H, d, CH₃-14), 0.85 (3H, d, CH₃-17) (Found: C, 49.6; H, 6.7; Ag, 17.8. $\text{C}_{26}\text{H}_{43}\text{O}_9\text{Ag}$ requires C, 51.4; H, 7.1; Ag, 17.8%).

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Example 2

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Liquid Formulation

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Example 3

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Ointment Formulation

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% w/w

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PEG 400

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PEG 4000

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Silver pseudominate

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The formulation may be produced by melting the mixture of PEG's and stirring in the silver pseudominate.

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Example 4

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Lotion Formulation

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% w/w

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PEG 400

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Ethanol

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Silver pseudominate

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Example 5

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Drop Formulation

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% w/w

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PEG 400

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Glycofurol

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Silver pseudomonate

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Example 6

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% w/w

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Cetomacrogol emulsifying ointment

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Polyethylene glycol 200

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Silver pseudomonate

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BIOLOGICAL DATA

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- a) The minimum inhibitory concentrations (MICs) of silver pseudomonate and sodium pseudomonate were determined against 20 strains of Pseudomonas aeruginosa in Blood Agar Base. Typical results are presented in Table 1. Silver pseudomonate was more active than sodium pseudomonate against all strains tested.
- b) MIC's of silver and sodium pseudomonate against various pathogenic bacteria were determined by standard methods. Typical results are presented in Table 2.

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Table 1

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The activity of Sodium Pseudomonate and Silver
Pseudomonate against 20 strains of
Pseudomonas aeruginosa:

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Typical MIC's

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Pseudomonas aeruginosa	MIC* ug/ml	
	Sodium Salt	Silver Salt
NCTC 10662	12,800	128
Dalglish	>128	128
PU7	>128	128
W985	>128	128
S41	>128	128
R60	>128	128
Pu4	>128	128
R59	>128	64
T3	>128	128
R3	6,400	128
R139	>128	128
R22	>128	128
W995	>128	128
59	>128	128
125	>128	128
4	>128	128
Fr13	6,400	128
D25	>128	128
ATCC 27853	>128	128
W996	>128	128

* MIC determined in serial dilution in Blood Agar
 Base. Inoculum of 0.001 ml of an overnight Tryptone
 Soya Broth Culture. Incubated at 37°C overnight.

Table 2Typical MIC's (µg/ml) against Human Bacteria

Organism	<u>Pseudomonte Salt, MIC (µg/ml)</u>	
	Silver	Sodium
E. coli NCTC 10418	128	125
P. mirabilis 889	128	125
K. aerogenes A	128	250
Ps. aeruginosa NCTC 10662	128	12800
Pasteurella multocida 1633	0.5	0.25
Haemophilus influenzae Wy21	0.12	0.12
Bacillus subtilis 6633	0.25	0.25
Corynebacterium xerosis 9755	128	>125
Staph. aureus Oxford	0.5	0.25
Staph. aureus Russell	0.5	0.25
Staph. aureus W2827	0.5	0.25
Strep. faecalis I	64	50
Strep. pyogenes R80/421-A	0.25	0.25
Strep. agalactiae 2788-B	1.0	0.5
Strep. spp. 64/848-C	1.0	0.5

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Claims

1. A compound silver pseudomonate.
2. A compound as claimed in claim 1, being at least 75% pure.
3. A compound as claimed in claim 1 or claim 2, being at least 90% pure.
4. A compound as claimed in any one of claims 1 to 3, being at least 95% pure.
5. A compound as claimed in any one of claims 1 to 4, being in the solvated form.
6. A compound as claimed in any one of claims 1 to 5, being in the hydrated form.
7. A process for producing silver pseudomonate which process comprises reacting silver ions and pseudomonic acid or pseudomonate ions in aqueous solution and thereafter recovering the silver pseudomonate so formed.
8. A process as claimed in claim 7, effected by adding a source of silver ions to an aqueous solution of pseudomonic acid or a pseudomonate salt.
9. A process as claimed in claim 7 or claim 8, wherein the pseudomonate salt is sodium pseudomonate.

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10. A process as claimed in claim 7 or claim 8, wherein the solution of pseudomonic acid or pseudomonate ions is the product of aerobically culturing Pseudomonas fluorescens (NCIB 10586).
11. A process as claimed in any one of claims 7 to 10, wherein the source of silver ions is a soluble silver salt.
12. A process as claimed in any one of claims 7 to 11, wherein the source of silver ions is silver nitrate.
13. A pharmaceutical formulation comprising silver pseudomonate and a pharmaceutically acceptable carrier therefor.
14. A pharmaceutical formulation as claimed in claim 13, formulated for topical application.
15. A pharmaceutical formulation as claimed in claim 13 or claim 14, comprising silver pseudomonate and at least 1% by weight of a poly (substituted or unsubstituted alkylene) glycol or a derivative thereof.
16. A pharmaceutical formulation as claimed in any one of claims 13 to 15, comprising polyethylene glycol or a derivative thereof.
17. A pharmaceutical formulation as claimed in any one of claims 13 to 16, comprising silver pseudomonate and at least 1% by weight of polyethylene glycol or a derivative thereof.

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- 01
02 18. A pharmaceutical formulation as claimed in any one
03 of claims 13 to 17, wherein silver pseudomonate is
04 the sole therapeutic agent.
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06 19. A compound as claimed in claim 1, for use in the
07 treatment of the human or animal body infected with
08 Pseudomonus organisms.
09 20. A compound as claimed in claim 19, for use in
10 treating infected wounds and burns infected with
11 Pseudomonus organisms.

0128338



European Patent
Office

EUR PEAN SEARCH REPORT

Application number

EP 84 10 5070

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 8)
A	EP-A-0 005 614 (BEECHAM GROUP) * Claims 1,2 *	1,7	C 07 D 407/06 A 61 K 31/35
A	EP-A-0 068 680 (BEECHAM GROUP)		
D, A	GB-A-1 395 907 (BEECHAM GROUP)		
			TECHNICAL FIELDS SEARCHED (Int. Cl. 8)
			C 07 D 407/06
The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 30-07-1984	Examiner PHILLIPS N.G.A.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			